Molecular Design of Human Modified β-Hexosaminidase B with Therapeutic Potential

Human lysosomal β-hexosaminidase A (HexA, αβ heterodimer) can degrade intralysosomal substrates including GM2 gangliosides (GM2) in cooperation with GM2 activator protein (GM2A). We produced a modified human HexB (modB) by CHO cells, composed of homodimeric β subunits with amino acid substitutions of RQNK (β312–315) and DL (β452–453) to the homologous α-type GSEP necessary for GM2A binding and NR for GM2 recognition. We designed and produced the mod2B with additional substitution of LDS (β316–318) to the α-type SGT based on the crystal structure of modB (PDB ID: 5BRO). We demonstrated that the GM2-degrading activity and therapeutic potential of mod2B are superior to those of modB for HexA deficiencies.

Lysosomal β-hexosaminidases (Hex) are hydrolases, which degrade terminal N-acetylhexosaminyl residues of glycoconjugates at acidic pH [1]. There are three human Hex isozymes: HexA (αβ heterodimer) [2], HexB (ββ homodimer) [3] and HexS (αα homodimer). Among them, only HexA can degrade GM2 ganglioside (GM2) in cooperation with GM2 activator protein (GM2A). In contrast, HexB prefers neutral substrates and exhibits high thermostability. The amino acid sequence identity between α and β subunits is about 57%, and both show very similar 3D structures.

Tay-Sachs disease (TSD) and Sandhoff disease (SD) are autosomal recessive HexA deficiencies, caused by *HEXA* and *HEXB* gene mutations, each encoding the human Hex α and β subunit, associated with excessive accumulation of GM2 in the brain and neurological symptoms [1]. Many heterogenous mutations including missense mutations causing misfolding of the mutant Hex isozymes have been reported. There are currently no effective treatments, although several therapeutic approaches have been developed, including intracerebroventricular enzyme replacement therapy (*icv*ERT) [4] and *in vivo* gene therapy [5]. In order to treat TSD and SD in patients, it should be crucial to restore HexA activity because only HexA is believed to degrade GM2. However, the HexA containing α subunit may be antigenic for TSD patients with Hex α-subunit deficiency. Therefore, we designed and produced human modified HexBs composed of homodimeric β subunits with GM2-degrading activity *in vivo* as well as putative low-antigenicity for TSD patients as "camouflaged" enzymes.

Two kinds of human modified HexB (modB and mod2B) were designed and produced by CHO cell lines, on the basis of X-ray crystal structures of HexA (αβ, PDB ID: 2GJX) [2] and HexB (ββ, PDB ID: 1NOU) [3]. The modB contains a partial amino acid sequence of the α subunit, i.e. the two substitutions of DL (β452–453) to NR (α423–424), necessary for anionic substrate recognition in the α subunit, as well as the RQNK (β312–315) to GSEP (α280–283), required for the interaction with GM2A, an essential cofactor for GM2 degradation. The modB is composed of the homodimeric modified

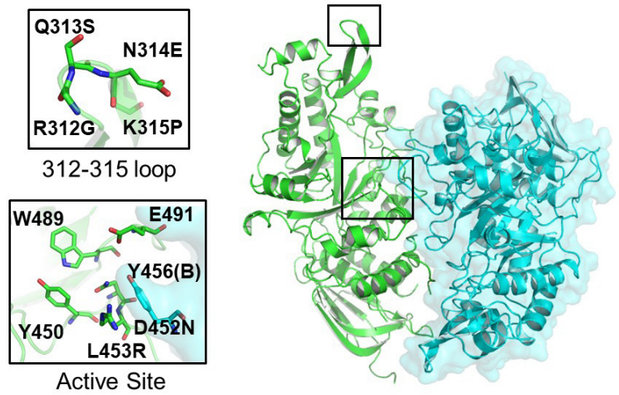

Figure 1: Crystal structure of human modB. Overall structure of the modB dimer determined at 2.4 Å. A close-up view of the modified β312–315 loop, and the active site residues are shown in insets. The electron densities of the omit map (contoured at 2σ) around the modified sites are also shown.

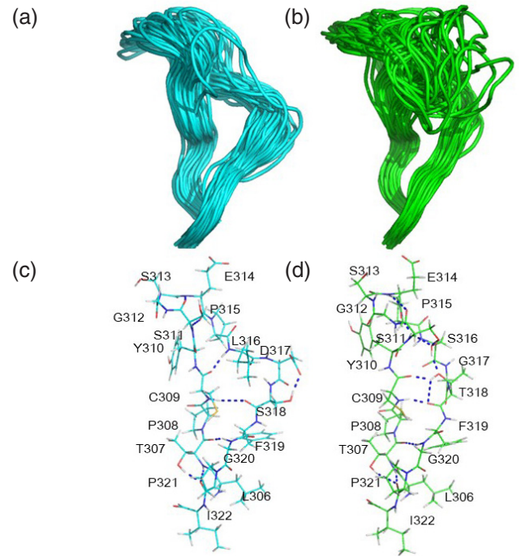

Figure 2: Predicted local models for human mod2B. **(a–d)** Structure of the GSEP loop region of modB and mod2B. Structural snapshots of the GSEP loop region of modB **(a)** and mod2B **(b)** from MD trajectories taken every 1 ns. The amino acid regions from 307–322, including the GSEP loop, are shown with ribbon models. Representative structures of the GSEP loop regions of modB **(c)** and mod2B **(d)** are shown as stick models. All nonpolar hydrogen atoms are omitted for clarity. Hydrogen bonds within the GSEP loop regions are depicted by blue dashed lines.

β subunits secreted by a CHO cell line, which retained thermostability similar to that of human HexB *in vitro*, and could reduce the accumulation of GM2 and GA2 in the brains of SD model mice following *icv* administration [4]. We elucidated the X-ray crystal structure of purified modB (PDB ID: 5BRO), which retained a local conformation of the active pocket of the modified β subunits very similar to that of the α subunits and the introduced GSEP loops (Fig. 1) [5]. (Supplemental material available online with the article at doi:10.1172/JCI85300DS1)

We further designed and purified the human mod2B, composed of the homodimeric precursor of modified β subunits, in which the LDS (β316–318) sequence was additionally replaced with SGT (α284–286) because it putatively protected the GSEP loop sequence from proteolytic degradation. We also predicted the structure of mod2B (Fig. 2) based on the crystal structure of modB (PDB ID: 5BRO) [5] and human HexA (PDB ID: 2GJX) [2]. The results of the trajectory analysis from a molecular dynamics (MD) simulation showed that the average pairwise root-mean-square deviation (RMSD) of the GSEP loop regions of modB and mod2B were 2.315 A and 3.869 A, respectively. S318 forms a hydrogen bond with D317 in modB, whereas S311, S316, and T318 form hydrogen bonds in the structural core area with S316, T318, and C309, respectively, in mod2B (Fig. 2, c and d). These MD simulation results suggest that the GSEP loop region is more flexible in mod2B than in modB.

We evaluated the therapeutic effects of *icv* injection of the modB and mod2B on SD mice at 10 weeks of age, which present an age-dependent onset of severe motor dysfunction. Following the *icv* administration (1–2 mg/kg body weight dose), the mod2B-treated SD mice exhibited more improved rota-rod performance and prolonged life-span than modB-treated mice [5]. These results showed that mod2B had more potent therapeutic availability than modB, likely due to the better resistance to proteases and the relatively longer half-life of GM2-degrading activity of mod2B in neural cells.

REFERENCES

[1] D. J. Mahuran, *Biochim. Biophys. Acta* **1455**, 105 (1999).
[2] M. J. Lemieux, B. L Mark, M. M. Cherney, S. G. Withers, D. J. Mahuran and M. N. James, *J. Mol. Biol.* **359**, 913 (2006).
[3] B. L. Mark, D. J. Mahuran, M. M. Cherney, D. Zhao, S. Knapp and M. N. James, *J. Mol. Biol.* **327**, 1093 (2003).
[4] K. Matsuoka, T. Tamura, D. Tsuji, Y. Dohzono, K. Kitakaze, K. Ohno, S. Saito, H. Sakuraba and K. Itoh, *Mol. Ther.* **19**, 1017 (2011).
[5] K. Kitakaze, Y. Mizutani, E. Sugiyama, C. Tasaki, D. Tsuji, N. Maita, T. Hirokawa, D. Asanuma, M. Kamiya, K. Sato, M. Setou, Y. Urano, T. Togawa, A. Otaka, H. Sakuraba and K. Itoh, *J. Clin. Invest.* **126**, 1691 (2016).

BEAMLINES

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